

REMARKS

This is meant to be a complete response to the Office Action mailed June 13, 2003, and a Summary of the Interview held between the Examiner and Applicants' representatives Kathryn Hester, Douglas Sorocco, and Mike Smith on October 7, 2003. In the Office Action, the Examiner stated the substitute Specification filed on 3/26/2003 was not entered because it did not conform to 37 C.F.R. 1.125(b). The Examiner also rejected Applicants' claims 1, 2 and 5-8 under 35 U.S.C. 112, ¶2, and rejected Applicants' claims 1, 2 and 5-8 under 35 U.S.C. 103(a) as being unpatentable over Prilliman et al. in view of Maniatis et al.

Substitute Specification

In the Office Action, the Examiner stated the substitute Specification filed on 3/26/2003 was not entered because it did not conform to 37 C.F.R. 1.125(b) because the statement as to a lack of new matter under 37 C.F.R. 1.125(b) was missing.

Applicants direct the Examiner's attention to Page 2, lines 8-11 of the Amendment filed on March 26, 2003:

Therefore, Applicant respectfully submits the corrections to the Specification are proper and necessary for a clear understanding of the invention claimed in the subject application, and that such corrections do not constitute new matter.

Therefore, Applicants respectfully submit the statement as to a lack of new matter was properly submitted on March 26, 2003, and thus request entry of the substitute Specification filed on March 26, 2003.

Applicants' Response to the 35 U.S.C. 112, ¶2 Rejection

In the Office Action, the Examiner rejected Applicants' claims 1, 2 and 5-8 under 35 U.S.C. 112, ¶2, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Examiner stated:

- A) Claims 1-2 and 5-8 are indefinite in their recitation of the phrase "isolating MHC allele mRNA" because it is not clear what is meant and the specification does not define MHC allele mRNA.
- B) Claim 8 is indefinite in its recitation of the phrase "wherein, in the step of isolating MHC allele mRNA from a source, the source is selected from the group consisting of a mammalian DNA specimen" because it is not clear how mRNA can be isolated from DNA.

In response to (A), claim 1 has been amended herein to recite "isolating total RNA from a source and reverse transcribing the mRNA to form cDNA, wherein the total RNA contains mRNA for at least one MHC Class I allele to form a cDNA encoding a desired MHC Class I allele". In response to (B), claim 8 has been amended herein to recite "the source is selected from the group consisting of a virus transformed cell line and an immortalized cell line".

Applicants respectfully submit the amended claims are now definite and particularly point out and distinctly claim the subject matter which Applicants

regard as the invention. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 112, ¶2 rejection of the claims as now amended.

Applicants' Response to the 35 U.S.C. 103(a) Rejection

In the Office Action, the Examiner rejected Applicants' claims 1, 2 and 5-8 under 35 U.S.C. 103(a) as being unpatentable over Prilliman et al. (Immunogenetics 45:379-385 (1997)) in view of Maniatis et al. (Molecular Cloning: A Laboratory Manual, second Edition, Cold Spring Harbor Laboratory, pages 129 and 191-192, 1982). Applicants respectfully traverse the rejection for the reasons stated hereinbelow.

The present invention, as recited in amended claims 1, 2 and 5-8 of the subject application, is directed to a method for large scale, continuous production of large quantities of an individual Class I MHC molecule. The method includes isolating **total RNA from a source** and reverse transcribing the mRNA to form cDNA, wherein the total RNA contains mRNA for at least one MHC Class I allele and reverse transcribing the mRNA forms a cDNA encoding a desired MHC Class I allele. A truncated PCR product encoding the desired MHC Class I allele is then created by PCR amplification, and the resulting PCR product does not encode the transmembrane and cytoplasmic domains of the desired MHC Class I molecule and thus encodes an individual, soluble MHC Class I molecule. The truncated PCR product is then cloned into a mammalian

expression vector and electroporated or transfected into a host cell, and the host cell is used to inoculate a hollow fiber bioreactor unit for large scale continuous production of the soluble individual Class I MHC molecule.

Prilliman et al. disclose large scale production of class I bound *peptides*. In the method of Prilliman et al., a **full-length, single stranded DNA clone of B*1501** was used as a template for PCR amplification with primers that truncate the expressed form of the molecule through removal of the transmembrane and cytoplasmic exons from the coding region. The PCR product was cloned into an expression vector and then used to transfect a lymphoblastoid cell line, which was then used to inoculate a bioreactor for harvesting sHLA and isolating sHLA-bound peptides.

Prilliman et al. use an ***isolated, single MHC allele clone*** as a starting material. Therefore Prilliman et al. do not teach, disclose or suggest starting with total RNA from a source and reverse transcribing the mRNA to form cDNA, wherein the total RNA contains mRNA for at least one MHC Class I allele and thus the pool of cDNA contains a cDNA encoding a desired MHC Class I allele, followed by PCR amplification of the cDNA encoding the desired MHC Class I allele from the total RNA (without first isolating the single, desired cDNA) to create a truncated PCR product encoding the desired individual, soluble Class I MHC molecule. In addition, Prilliman et al. only teach one MHC allele and do

not teach, disclose or suggest how their method can be adapted for use with other desired MHC alleles, including how to obtain other MHC alleles.

The Examiner has recognized the deficiencies of Prilliman et al. and has attempted to supply the deficiencies with the teachings of Maniatis et al. While it is agreed that Maniatis et al. teach the isolation of mRNA from mammalian cells and the use of reverse transcriptase to transcribe mRNA into cDNA, it adds nothing to the fact that Prilliman et al. do not teach, disclose or suggest PCR amplification of *a single cDNA from a pool of cDNA obtained from total RNA, without first isolating and cloning the single, desired cDNA,* to create a truncated PCR product encoding the desired individual, soluble Class I MHC molecule. The combination of Prilliman et al. and Maniatis et al. does not teach, disclose or even suggest PCR amplification of a desired allele from cDNA reverse transcribed from mRNA present in a total RNA sample, and especially does not teach, disclose or even suggest locus-specific PCR amplification of MHC alleles. Further, the combination of Prilliman et al. and Maniatis et al. do not teach, disclose or even suggest the combination in a **single** PCR step of locus-specific amplification of MHC alleles with truncation to provide a PCR product encoding a soluble MHC molecule.

Locus-specific amplification of MHC alleles (i.e., PCR amplification of a desired MHC allele from cDNA reverse transcribed from mRNA present in a total RNA sample), in combination with PCR truncation, is an important and non-

obvious step of the present invention, because multiple MHC alleles are present in total RNA from a mammalian source. These multiple MHC alleles exhibit extensive cross-reactivity as well as high sequence similarity. Therefore, extensive care must be taken to prepare primers that will only amplify one of the MHC alleles present in a sample; otherwise, the PCR product will be a **mixture** of more than one MHC allele.

One of ordinary skill in the art will recognize that starting with an isolated, purified allele clone (i.e., a single strand of DNA as taught by Prilliman et al.) is completely different than starting with cDNA obtained from total RNA of a mammalian cell line, and neither Prilliman et al. nor Maniatis et al. provide any teachings or suggestions on how to amplify a **single** MHC allele from total RNA of a mammalian cell line and prevent amplification of **multiple** MHC alleles in the same reaction. Therefore, a person of ordinary skill in the art would not find the steps of MHC locus-specific amplification combined with truncation to provide a PCR product encoding a single, soluble MHC molecule in a **single** PCR step, as recited in the claims of the subject application, obvious over the combination of Prilliman et al. and Maniatis et al.

Therefore, Applicants respectfully submit that claims 1, 2 and 5-8 are non-obvious over the combination of Prilliman et al. and Maniatis et al. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 103(a) rejection of claims 1, 2 and 5-8 over such combination of references.

Summary of the Interview

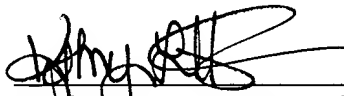
A personal interview was held on October 7, 2003, between the Examiner and Applicants' representatives Kathryn Hester, Douglas Sorocco, and Mike Smith. In the Interview, claims 1, 2 and 5-8 were discussed, as was the Prilliman et al. reference. In the Interview, Applicants' representatives discussed possible amendments to the claims in order to differentiate the invention from the cited reference.

CONCLUSION

This is meant to be a complete response to the Office Action mailed June 13, 2003 and a Summary of the Interview held on October 7, 2003. Applicants respectfully submit that claims 1, 2 and 5-8, as now amended, are patentable over the art of record. Further, Applicants respectfully submit that newly added claims 9-14 are also patentable over the art of record. Favorable action is respectfully solicited.

Should the Examiner have any questions regarding this Amendment, or the remarks contained herein, Applicants' agent would welcome the opportunity to discuss such matters with the Examiner.

Respectfully submitted,



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